ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 14, Issue, 11, pp.22882-22885, November, 2022
DOI: https://doi.org/10.24941/ijcr.44298.11.2022

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

RESISTANCE PROFILE OF CANDIDA ALBICANS STRAINS ISOLATED AT THE NATIONAL PUBLIC HEALTH LABORATORY IN BRAZZAVILLE

MOUNKALA P. Christoffer[1,2*], MOYEN Rachel[2], BIDOUNGA Joël[2], MAKAYA Nicole[1] and NIAMA Fabien[1,2]

[1]National Public Health Laboratory, Department of Microbiology; [2]Cellular and Molecular BiologyLaboratory, Department of Biology, Faculty of Science and Technology, Marien Ngouabi University, BP.69 brazzaville, Congo

ARTICLE INFO

Article History:
Received 18th August, 2022
Received in revised form
24th September, 2022
Accepted 15th October, 2022
Published online 30th November, 2022

Key words:

Vaginal swab; Candida albicans; Antimycotics; Resistance Profile.

Corresponding Author:
MOUNKALA Christoffer

ABSTRACT

Objective: The objective of this study is to evaluate the prevalence and resistance patterns of *Candida albicans* strains isolated from vaginal swabs at the National Public Health Laboratory in Brazzaville. ***Material and methods:*** 298 vaginal swabs were collected at the National Public Health Laboratory from January to June 2020. Isolation was performed on Sabouraud's medium, followed by species identification based on phenotypic and biochemical tests. Susceptibility to antifungal agents was assessed by standard solid and liquid diffusion techniques according to CLSI/NCCLS 2009-2020. ***Results:*** Mycological examination of the 298 samples revealed 99 positive results, of which 37 were identified as *Candida albicans,* a prevalence of 37.37%. Of the 37 strains of *C. albicans* tested, 19 were resistant, i.e. 51.35%, according to the antifungal activities of the strains. The species had a comparatively high level of resistance to azoles, including miconazole (47.94%) and fluconazole (27.09%). Four main resistance patterns were identified from the data analysis: 14 strains showed AT pattern (azoleresistance), 3 strainsshowed AP pattern (imidazole-polyeneresistance), 1 strain showed PAT pattern (polyene-azoleresistance) and 1 strain showed PF pattern (polyene-5flurocytosine). ***Conclusion:*** This study found that among the species of the genus *Candida*, *Candida albicans* has the highest prevalence. Through the study of antimycoticactivities, we were able to follow the evolution of the resistance of the species to azoledrugs, which constitutes a serious threat to public health.

Citation: ***MOUNKALA P. Christoffer, MOYEN Rachel, BIDOUNGA Joël, MAKAYA Nicole and NIAMA Fabien.*** **2022.** "Resistance profile of candida albicans strains isolated at the national public health laboratory in brazzaville*". International Journal of Current Research*, 14, (11), 22882-22885.

INTRODUCTION

The majority of common fungal infections, including vulvovaginitis, are caused by the genus *Candida*, with 75% of women likely to experience at least one episode of this infection in their lifetime (Hédon, 1998) (FPaS, 1996). In the sexually active female population, the incidence of fungal infections is in the range of 10-15%. This incidence appears to have increased threefold over the past 15 years (Lansac Plchm, 1998). *Candida albicans* is the most widely researched species in this group to date. It is present in the vagina in a saprophytic state in 10% of women, but its prevalence is higher in 5-15% of pregnant women and in women using oral contraceptives. In addition, surveys indicate that 25-50% of women are asymptomatic vaginal carriers of Candida albicans (Hédon, 1998). Due to its prevalence and severity, this species causes some of the most typical fungal infections (Bodey, 2002; Samaranayake, 2002). The most typical infection is commensal *Candida albicans* in the vagina. It has a special place among fungal infections because it affects many healthy women of childbearing age (Sobel, 1998). The less obvious signs of vaginal candidiasis are minimal or abundant whitish leucorrhoea with a granular appearance and often intense vulvar pruritus. Although the clinical symptoms are indistinguishable from other diseases, vaginal secretions and smears can be used to make the diagnosis. However, a microbiological diagnosis is necessary to demonstrate the presence of yeast (Landers, 2004).

The diagnosis can be quickly guided by direct examination of vaginal swabs. Misdiagnosis on the basis of direct examination is corrected by culture, including media. Species identification using phenotypic, biochemical and molecular criteria is necessary for appropriate treatment. The objective of this study is to identify the prevalence and resistance profiles of *Candida albicans* strains that have been isolated from vaginal candidiasis at the National Public Health Laboratory in Brazzaville.

MATERIAL AND METHODS

MATERIAL

Mainly vaginal swabs from samples collected at the National Public Health Laboratory.

METHODS

Sampling: A descriptive cross-sectional study was carried out on 298 samples ranging in age from 17 to 60 years, of which 99 were mycologically positive and were kept at the National Public Health Laboratory from January to July 2020.

Sample analysis

Mycological examination: A classic mycological examination was carried out on each sample according to standard microbiological techniques for the detection of yeasts, i.e. a direct examination to observe the fungal elements on the slide, and culture on Sabouraud medium with chloramphenicol at 37 C° for 24 to 48 hours.

Phenotypic identification

Morphological and physiogical identification: A rapid diagnosis of Candida albicans species, was performed on the basis of morphological, physiological and cultural criteria obtained from various analyses, including observation of isolated colonies on culture media, serum filamentation test and rat media (Chabasse, 1999).

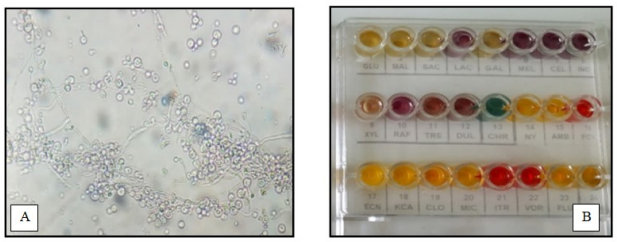
Biochemical identification: The Integral yeasts plus liofilchen system is used for species identification. With this kit, it is possible to identify yeasts of clinical interest and assess their sensitivity to antifungal agents. The kit has a total of 24 wells, 13 of which allow the identification of up to 32 different yeast species and 11 of which allow the testing of sensitivity to antifungal agents. Based on sugar uptake reactions, the identification principle interprets the uptake reaction tests by analysing the colour change of the wells from 1-GLU to 12-DUL. The combination of positive and negative reactions creates a numerical code that will allow the identification of the yeast being examined. The 13-CHR well contains a chromogenic substrate that directs the differentiation of particular yeasts by evaluating the colour change of the well, thus confirming the identification made (Diagnostic).

Study of the resistance profile: Antifungal susceptibility was assessed using two methods: the first was performed using the agar diffusion technique according to M44-S3 and M44-A2 CLSI/NCCLS 2009 on the interpretation of disc lysis zones at specific concentrations (see Table 1) and the second with the Integral yeasts plus liofilchen system designed according to M27-A2 CLSI/NCCLS2020 on antifungals of clinically isolated fungal species, The principle is based on the growth or inhibition of yeasts in wells containing specific concentrations of antifungal agent plus a pH indicator. The colour change in the wells indicates the growth of the yeast examined (Susceptible: red, Intermediate or SDS: orange, Resistant: yellow); well 24 is called the control well and contains only a standard yeast culture medium and a pH indicator (Diagnostic).

RESULTS

Sampling characteristic: The following table shows how the genital samples were distributed among the women, whose ages ranged from 15 to 70 years. (see Table 2)

PREVALENCE OF ISOLATED CANDIDA SPECIES



Legend: Image A shows the identification of C. albicans on the gallery integral yeast plus system used in this study; Image B shows the observation of C. albicans in its fresh state following filamentation testing at a magnification of X400 under a light microscope.

Figure 1. Sampling characteristic

Prevalence of isolated candida species: A total of 298 vaginal swabs were submitted for this study, of which 99 (33.22%) were found to be mycologically positive. After performing serum and RAT filamentation tests of the isolated species, it was found that 37.37% of the isolates were *Candida albicans*. **(**see table 3)

Variation in the prevalence of candida species according to patient age.

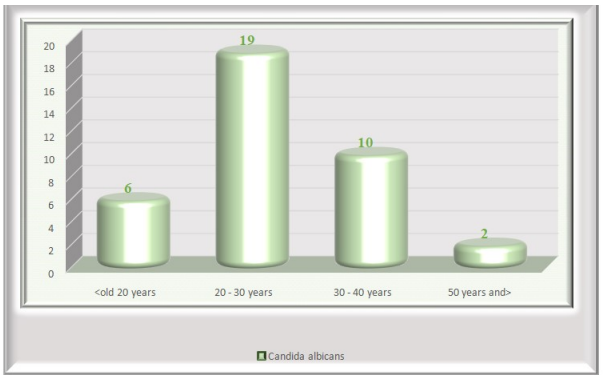


Figure 2: Age-specific variation in prevalence

STUDY OF SENSITIVITY TO ANTIMYCOTICS

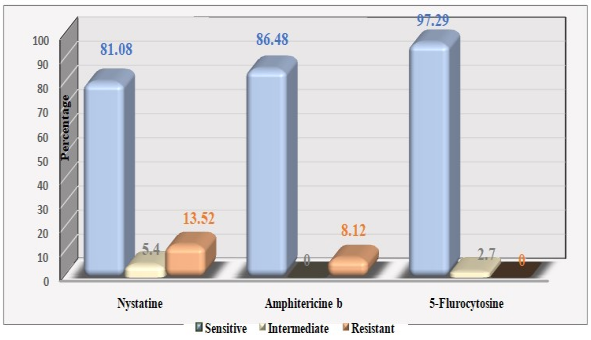


Figure 3: Rate of sensitivity of species to polyene and 5-fluorocytosine

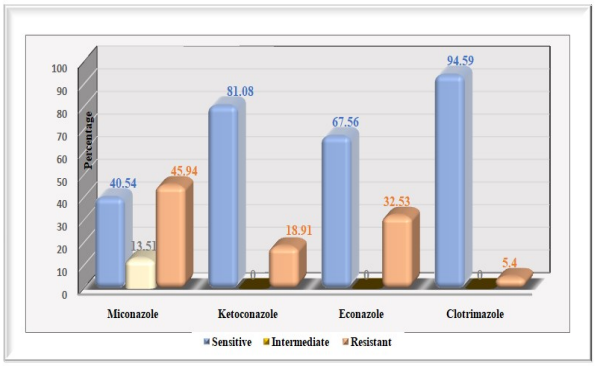


Figure 4: Rate of sensitivity of species to imidazole

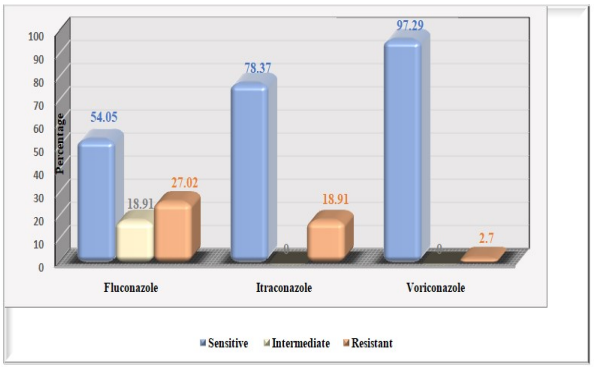


Figure 5. Rate of sensitivity of species to triazole

Variation in the prevalence of candida species according to patient age: After identification on the yeast plus gallery of the Integral system, a study of the prevalence according to the age of the patient revealed that the prevalence was higher in the 20-30 age group and lower in the over 50 age group.

Resistance rates to miconazole and econazole on these species were quite high, approaching 50%, reflecting the antimycotic activity of imidazoles. Fluconazole has a significant dependent or intermediate susceptibility and a resistance of 27.02%, which distinguishes the susceptibility rates of the triazoles of the species.

Table 1. Table 1 : Antifungigram Interpretation

			the acceptable range for inhibition zone diameters in millimeters			Galerie integral system yeast CMI en µg/ ml		
Designation	Code	Charge	S	SDD	R	S	SDD	R
Nystatin	NYS	50 µg	≥ 15	14 - 10	≤ 10		-	>1,25
Amphotericin B	AMB	10 µg	≥ 15	14 - 10	≤ 10	< 2	-	≥ 2
Voroconazol	VOR	1 µg	>17	14 - 16	≤ 13	≤ 1	2	≥ 4
Clotrimazol	CLO	50 µg	> 20	20 - 10	= 10	< 1	-	≥ 1
Econazol	ECO	50 µg	> 20	20 - 10	= 10	< 2	-	≥ 2
Fluconazol	FLU	25 µg	> 19	18 - 15	≤ 14	< 8	16-32	> 64
Itraconazol	ITR	10 µg	> 23	22 - 14	≤ 13	<0,125	0.25-0.5	>1
Miconazol	MIC	50 µg	> 20	20 - 10	= 10	< 2	-	≥ 2
Ketoconazol	KCA	50 µg	> 20	20 - 10	= 10	≤ 0,25	-	≥ 0,5
Fluorocytosin	5-FC	1 µg	≥ 20	12 - 19	≤ 11	< 4	8-16	≥32

Table 2: Sampling

Age group	*Frequency*(N= 298)	*Percentage* (%)
Under 20 yearsold	26	8,72
20 - 30 yearsold	55	18,45
30 - 40 yearsold	130	43,62
50 years and over	70	23,48

Table 3. Prevalence of *Candida* species isolated

	Frequency N= 298	*Percentage*	*IC(95%)*
Positive	99	33,22	28,7-38,5
Negative	199	66,77	61,4-72,1
The main species identified by the explosion test			
Candida albicans	37	37,37	32,77-42,86

Study of the sensitivity to antimycotics: The susceptibility of *C. albicans* strains to antimycotics showed a variation in susceptibility rates according to the families of molecules tested.

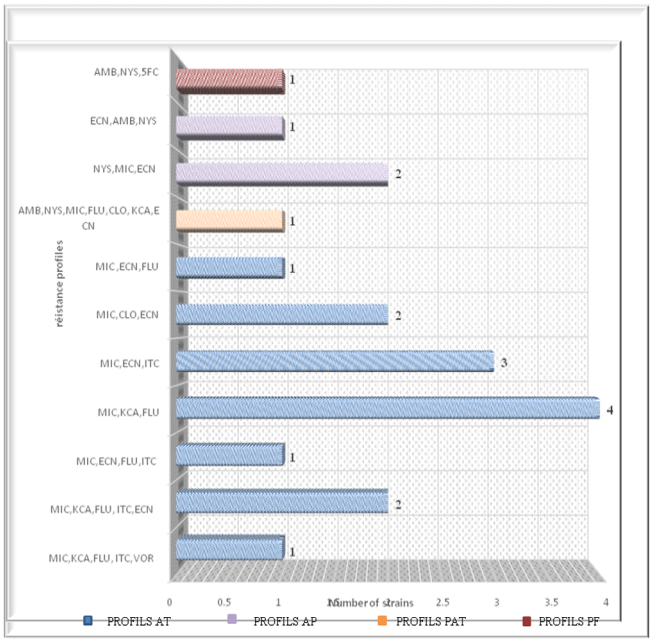


Figure 6. Profiles of resistance found

The sensitivity rates of amphotericin B and nystatin to polyenes were 83.74% and 81.09% respectively, while the sensitivity rates of azoles ranged from 59.46% to 100%.

DISCUSSION

With a prevalence of 37.37%, *Candida albicans* species remains dominant over the other species as described by Nsabimana et al, Richter et al, 2007. However, this result differs from the results obtained by Sylla K Sd 2017 (Sylla, 2017) on the prevalence of vulvovaginal candidiasis, which presented a figure of 71.51% for *C. albicans*. The study of the antimycotic activities of the species by applying the two methods, that of diffusion on solid medium CLSI/NCCLS 2009 and that of dilution on liquid medium M27-A2 CLSI/NCCLS2020, confirmed the similarity of the two methods, as no significant difference was found between the two methods. The data obtained on the sensitivities of the species to polyenes confirmed the low resistance of the species to these molecules, with sensitivities of 81.08% and 86.48% for amphytericin B and nystatin, which correlate with those obtained by several researchers on this species (Sylla, 2017; Djohan, 2011). Resistance to 5-fluorocytosine was low (2.7%), which confirms the results obtained by other authors (Sandra, 2005; Yapo-Kouadio, 2017) . The results obtained on azole activity were characterised by high levels of resistance to miconazole 45.94%, Econazole 32.42% and Fluconazole 27.02; this differs from the results of Amouri (Amouri, 2010) who described *C. albicans* as generally susceptible to azoles, these results correlate with the study of YAPO KOUADIO (Yapo-Kouadio, 2017) and Djohan (Djohan, 2021), which showed that in nine (9) years the resistance of *C. albicans* to fluconazole increased from 2.2% to 23.4%. The target of azoles is the ergosterol biosynthetic pathway by blocking C14 a demethylase (CYP51 encoded by the ERG11 gene), which is responsible for the conversion of lanosterol to ergosterol. Azoles cause an accumulation of toxic methylated sterols and membrane damage. *C. albicans* and other species of the genus can resist azoles by overproduction or modification of the ERG11 gene, by activation of efflux pumps whose

function is to remove toxic substances from cells, or by metabolic bypass using an alternative metabolic pathway or by mutationally blocking the biosynthetic pathway that leads to the toxic metabolite (20). The resistance observed in practice may result from an accumulation of independent genetic events.

CONCLUSION

Although it is the most virulent species in the genus, *C. albicans* is the least common in the clinical setting (Fadda). The results of this study demonstrated the importance of keeping an eye on the microbiological resistance patterns of this species in light of the increasing resistance to azole drugs. As the latest generation of antimycotic molecules are expensive and require long antimycotic treatments, the increase of this resistance is a serious threat to public health. These results support the idea that misuse of antimycotics could be one of the factors contributing to the resistance observed in this study, but they also show that only the azoles most frequently prescribed in our country to treat vaginal candidiasis show high rates of resistance.

REFERENCES

Hédon B. DD, P. Madelenat, Frydman. S: " gynécologie". Paris: Ellipses; 1998.

FPaS: JD Immunopathogenesis of recurrentvulvovaginal candidiasis. *Clin Microbiol* 1996, Rev. 9: 335—348.

LANSAC PLCHM, J. " Gynécologie pour le praticien " Paris: Masson; 1998, 2002.

Bodey GP, Mardani, M., Hanna, H. A., Boktour, M., Abbas, J.,Girgawy,E., Hachem, R.Y., Kontoyiannis, D. P. and Raad, II: The epidemiologyof Candida glabrataand Candida albicans fungemia inimmunocompromised patients with cancer. *Am J Med* 2002:112: 380—385.

Samaranayake LP, Fidel, P. L., Naglik, J. R., Sweet, S. P., Teanpaisan,R., Coogan, M. M., Blignaut, E. and Wanzala: Fungal infectionsassociated with HIV infection. Oral Dis. 2002:8 Suppl 2: 151—160.

Sobel J: Vulvovaginitis due to Candida glabrata an emerging problem. *Mycoses Probl* 1998:41:18—22.

Landers DV WH, Heine RP, et al: Predictive value of theclinical diagnosis of lower genital tract infection in women. *Am J ObstetGynecol* 2004:190, 1004—1010.

Chabasse D GC, Contet–Audonneau N: MycologieMédicale. Paris: Masson; 1999.

1995 KH: Guide de mycologie médicale. . In. Edited by Ellipses S; 1995.

Diagnostic L: Integral systeme yeast plus In: *https:// wwwli of ilchemcom/ solutions /clinical/microbial-i dentification/id-systems/id-ast-systems-pure-colony.*al Re: Antifungal susceptibilities of Candida species causing vulvovaginitis and Epidemiology of recurrent cases. *J Clin Microbiol* 2005:43 : 2155 - 2165.

Nsabimana JD TM, ClaeysG, Verschraegen G: Détermination de la sensibilité de 45 souches de Candida au uconazole : Analyse de deux tecniques de référence. *Med afr noire* 2007:34 - 40.

SYLLA K SD, LAKHE N.A, TINE R.C, DIA M, LELO S, AHAMADA H.M, FAYE B,: Candidoses vulvo-vaginales au laboratoire de Parasitologie-Mycologie du Centre Hospitalier Universitaire de Fann, Dakar (Sénégal). *RevCames sante* 2017:21 - 27.

Djohan V KEA, A. H. Vanga-Bosson et coll: sensibilité in vitro des souches de Candida albicans d'origine vaginale aux antifongiques à abidjan (Côte d'Ivoire). *J Mycol Med* 2011:22, 129-133.

Sandra SR RP, Shawn AM, Richard JH, Daniel JD, Michael A: Antifungal susceptibilities of Candida Species causing vulvovaginitis and epidemiology of recurrent cases. *J Clin Microbiol* 2005:43, 2155-2162.

GJeD: E La Résistance Aux Antifongiques : Importance En Médecine Humaine Et Vétérinaire. *Bull Acad Vét* 2015:Tome 168, 164, 314-319.

CG YAPO-KOUADIO AB-I, NAD AKA, GSP DOU, KD ZIKA, S CISSÉ, KD ADOUBRYN, M DOSSO: Resistance of Vaginal Candida Albicans to Antifungal Drugs in Abidjan (Cote d'Ivoire). *Revue Bio-Africa* 2017:7-12.

Amouri I AS, Sellami H, Makni F, Sellami A, Ayadi A: La Candidose vulvovaginale: revue. *J Mycol Med* 2010: 20,108-115.

CG YAPO-KOUADIO AB-I, NAD AKA, GSP DOU, KD ZIKA,S CISSÉ3, KD ADOUBRYN , M DOSSO: PROFIL DE RÉSISTANCE DE CANDIDA ALBICANS D'ORIGINE VAGINALE AUX ANTIFONGIQUES USUELS À ABIDJAN, (CÔTE D'IVOIRE) *Revue Bio-Africa - N° 16* 2017:pp. 7-12.

D S. Resistance of Candida species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect Dis* 2002:2: 73-85.

Fadda M. E. GSP, M. B. Pisano, M. Deplano, and S.Cosentino: "Prevalence of Candida species in different hospitalwards and their susceptibility to antifungal agents: results of athree year survey. *Journal of Preventive Medicine and Hygiene,vol.*

****